EVALUATION OF ANTIUROLITHIC AND ANTIOXIDANT ACTIVITIES OF *Glochidion velutinum* LEAVES

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI - 32.

In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

Name: ASHMI RAHIM

REG.No. 261525203

Under the Guidance of

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Komarapalayam – 638183.

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Internal Examiner **External Examiner**

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DECLARATON

I hereby declare that the dissertation **"Evaluation of Antiurolithic and antioxidant activities of *Glochidion velutinum* leaves"**, has been carried out under the guidance and supervision of Mr.V.Venkateswaran, M.Pharm., Assistant Professor, Department of Pharmacology, J.K.K.Nataraja College of Pharmacy. Komarapalayam, in partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmacology during the academic year 2016-2017. I further declare that, this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma associateship and fellowship or any other similar title.

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Dedicated to

Parents,

Teachers&

My Family

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1. INTRODUCTION

1.1. Kidney Stones

The incidence of kidney stones has been increasing in western societies in the last five decades, in association with economic development. Most calculi in the urinal system arise from a common component of urine, e.g. calciumoxalate (CaOx), representing upto 80% of analyzed stones. Currently, open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of Extracorporeal Shock Wave Lithotripsy (ESWL), which has revolutionized urological practice and almost become the standard procedure for eliminating kidney stones.

However, in addition to the traumatic effects of shock waves, persistent residual stone fragments and the possibility of infection, suggest that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence (Begun, F.P. 1991). Urolithiasis is still a mysterious disease even after extensive research in urology. Sophisticated instruments, investigation etc., have failed to trace out the exact mechanism of urolithiasis, but they are manifesting this condition. The treatment in modern medicine is not only expensive but also not easily affordable to the needy poor. Actually there is no satisfactory drug in modern medicine which can dissolve the stone and the physician remains to be depending on alternative systems of medicine for better relief.

Herbal medicines are efficacious and have lesser side effect compared to modern medicines and also reduce the recurrence rate of renal stone Although the complete mechanism of action of these remedies are lacking but, plant based phytotherapeutic agents represent the chiefity used in medicine for urolithiasis. Unlike allopathic medicines which targets only one aspect of urolithiatic pathophysiology, most of the plant based therapy have been depictn to be effective at different stages of stone pathophysiology. The plant based drugs exerts their antilithogenic property by altering

the ionic composition of urine i.e. decreasing the calcium ion concentration or increasing the magnesium and citrate excretion. These remedies also express diuretic effect or lithotriptic activity. Drug with multiple mechanisms of protective action may be one way forward in minimizing tissue injury in human. Herbal medicines have several phytoconstituents and exert their beneficial effects in urolithiasis by multiple mechanisms.

1. Helps in spontaneous passage of calculi by increasing urine volume, PH and anti-calcifying activity.
2. Balance the inhibitors and promoter of the crystalisation in urine and effects the crystal nucleation, aggregation and growth(crystallisation inhibition activity)
3. Relieves the binding mucine of calculi (lithotriptic activity)
4. Improve renal functions.
5. Regulate oxalate metabolism.
6. Regulate the crystalloid colloid imbalance and improve renal function, thus prevents recurrence of urinal calculi
7. Improve renal tissue antioxidant status ans cell membrane integrity and prevent reoccurrence (Antioxidant activity)
8. Exerts noteworthy anti-infective action in aligned with the chief causative organisms (antimicrobial activity).
9. Reveals marked improvement in symptoms of urinal calculi like pain, burning micturation and haematuria (Analgesic and anti-inflammatory activity)

In Herbal treatment of kidney stones, drugs used to dissolve the stone or aid their passing to guard aligned with further retention. Diuretic action is also needed to increasing the amount of fluid going through the kidneys and flush out the deposits (Arafat,OM, 2008). Lithotripsy means breaking and disintegrating or dissolution of the preformed stones. Some of the drugs increasing the urine volume decreasing the saturation of the salts and prevents the precipitation of the crystals at physiological pH. Some of the herbal drugs disaggregate mucoproteins, which actually binds the crystal to the renal cells (Atmani.F.2003). Stones occur when urinal chemistry consequences in increase concentrations of stone salts (Oxalates, Calcium, Phosphates) that leads to super-

saturation (SS) and exceeds the limit of metastability for that salt in solution.Increased urine volume decreases the saturation of the salts and prevents the precipitation of the crystal at physiological pH. All herbal medicines used for the treatment of the urolithiasis also have diuretic action and some are known to alkalize the urine.

Inhibitors are defined as molecules that increase the Super Saturation (SS) required to initiate nucleation, decrease crystal growth rate and aggregation, and inhibit secondary nucleation. In contrast promoters reduce the formation product of the supersaturated solution. Some of the common promoters are oxalate, calcium, cystine, uric acid and inhibitors are citrate and magnesium. An imbalance between urinal-promoting and inhibiting factors has been suggested as more important in urinal stone formation than a disturbance of any single substance(adhirai.M 1997). An assortment of physiological inhibitors of urolithiasis found in urine including inorganic (e.g., magnesium) and organic (e.g., Citrate, Urinal prothrombin fragment, Glycosaminoglycans (GAGs) and other macromolecule) substances are known to inhibit stone formation. Organic inhibitory compounds adsorb to the surface of the crystal, thereby inhibiting crystal nucleation, growth and aggregation. Interference with crystal growth and aggregation therefore seems a possible therapeutic strategy for the prevention of recurrent stone disease. The medicinal plants contain chemical compounds like Glycosaminoglycans (GAGs) which themselves possess an inhibitor effect in the crystallization of calcium oxalate. Macromolecule of higher molecular weight of plant extract excerpts their action similar to natural urinal inhibitors and inhibits crystal.

In urine there are a number of crystalloids of different types (oxalate, uric acid, calcium, cystine) which are kept in solution by the presence of colloids (mucin and sulphuric acid) in the urine by the process of absorption. When there is imbalance in the crystalloid-colloid ratio, i.e., increase in crystalloid and fall in colloid level leading to formation of renal stones or when the colloid lose the solvent action or adhesive property, urinal stones are formed.In this condition the Glomerular Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinal system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid

get accumulated in blood. Herbal therapy improves the renal function by increasing the excretion of urea and creatinine. Most of the phytothereupatic agent exerts their antiurolithiatic effect through this mechanism given.

Hyperoxaluria is a most noteworthy risk factor in the pathogenesis of renal stone. It has been perceived and documented that oxalate play an important role in stone formation and has about 15-fold greater effect than urinal calcium. Increased oxalate concentration is responsible for precipitation and deposition of CaOx crystals. Herbal extract interfere with the metabolism of oxalate in male rats fed sodium glycolate. Glycolate feeding consequenceed in hyperoxaluria as well as increased activities of oxalate synthesizing enzymes of the liver i.e., glycolate oxidase (GAO), glycolate dehydrogenase (GAD) and lactate dehydrogenase (LDH), and lessened kidney LDH activity. Increased excretion of phosphorus has been perceived and documented in stone formers. Increased urinal phosphorus excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which induces calcium oxalate deposition . Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of GAGs. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Supersaturation of these urinal colloids consequences in precipitation as crystal initiation particle which when trapped acts as a nidus leading to subsequent crystal growth and Cystone (polyherbal formulation) maintain crystalloid-colloid balance by decreasing excretion of urinal calcium, oxalate, uric acid, phosphorus and protein in urolithiasis.

In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of aqueous and alcoholic extract of *Glochidion velutinum* using ethylene glycol induced hyperoxaluria model in rats and some relevant symptoms like anti-analgesic, anti-inflammatory, antioxidant and antimicrobial activity studies also validated.

Fig . No:1 : Functions of Kidney

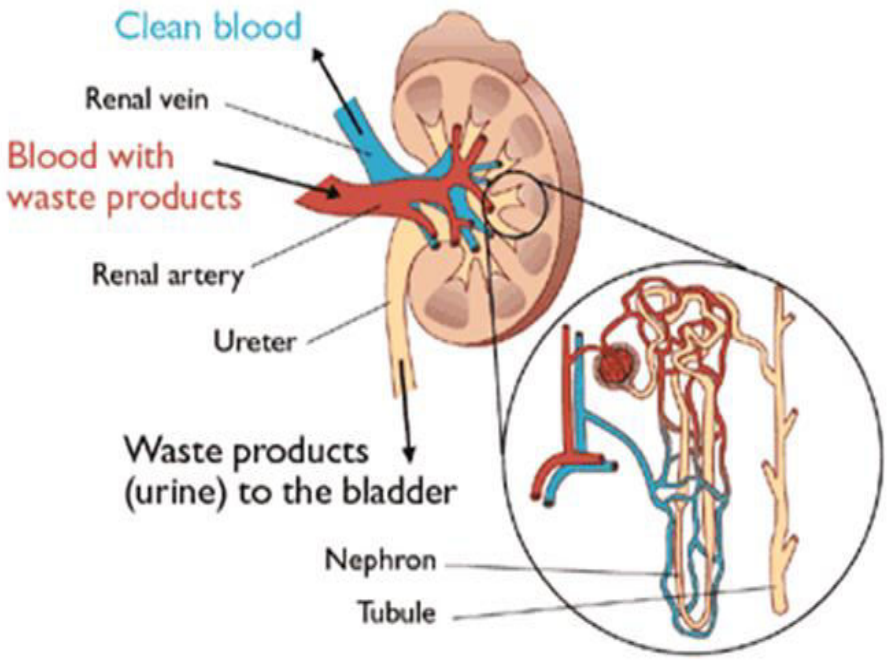
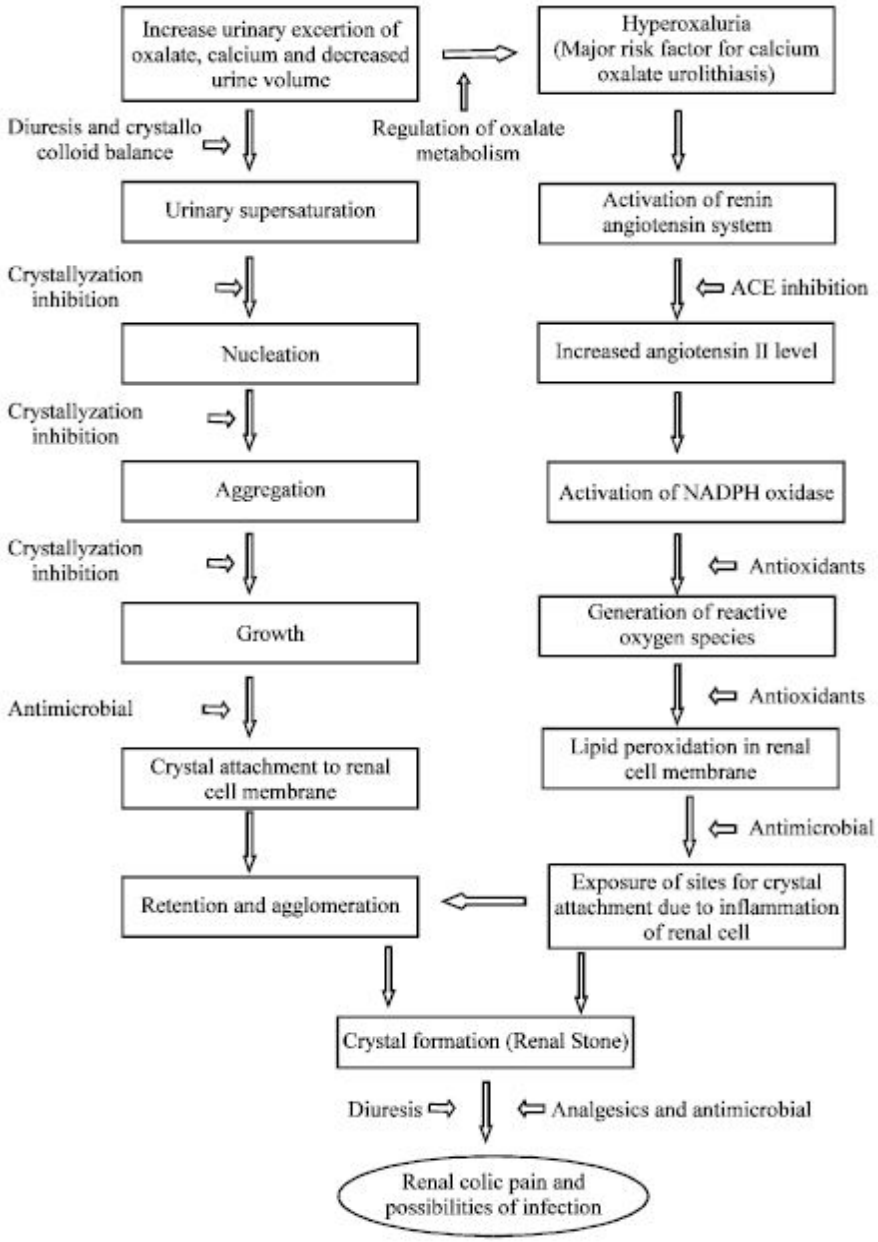


Fig . No: 2: Mechanism of Kidney Stone Formation



1.2. Classification of renal stones

Kidney stones may contain an assortment of combination of chemicals. The four most common types of kidney stones contain (Mukharjee.T. et al 1984)

- Calcium
- Struvite
- Uric acid
- Cystine

1.3. Aetiology of stone formation

An etiology of the urinal calculi is by no means clear, but the following possible factors may be considered.

- Stones can be classified into those caused by infection, or non-infectious causes, genetic defects or adverse drug effects (drug stones)
- **Non-infection stones**
  - Calcium oxalate
  - Calcium phosphate (including brushite and carbonate apatite)
  - Uric acid
- **Infection stones**
  - Magnesium ammonium phosphate
  - Carbonate apatite
  - Ammonium urate
- **Genetic causes**
  - Cystine
  - Xanthine

  2, 8-dihydroxyadenine
- **Drug stones**
- **Dehydration**
- **p^H of the urine**
- **Concentration of urinal salts**

- **Vitamin a deficiency**
- **Parathyroid hormone**
- **Prolonged immunity**
- **Nephrocalcinosis**

1.4. Stone composition

Metabolic aspects are important in stone formation, and metabolic evaluation is required to rule out anydisorders. Analysis in relation to metabolic disorders is the basis for further diagnostic and managementdecisions. Stones are often formed from a mixture of substances.

Most renal calculi contain calcium, usually in the form of calcium oxalate (CaC_2O_4) and often mixed with calcium phosphate ($CaPO_4$). In most instances no specific cause can be identified, although most patients have idiopathic hypercalcuria without hypercalcaemia.

Brushite is a unique form of calcium phosphate stones that tends to recur quickly if patients are not treated aggressively with stone prevention measures and are resistant to treatment with shock wave lithotripsy.

Interestingly hyperuricosuria is also associated with increased calcium containing stone formation, and is thought to be related to the uric acid crystals on which calcium oxalate and calcium phosphate can precipitate.

Rarely the underlying cause is primary oxaluria a liver enzyme deficiency leading to massive medullary nephrocalcinosis and renal failure.

Small asymptomatic stones in the kidney can be safely ignored, and if patients maintain good states of hydration, the risk of recurrent symptoms can be dramatically abridged. In all settings a search for a possible underlying cause of hyperoxaluria/hypercalcuria should be sought and if present corrected when possible.

1.5. Struvite stones

Struvite (magnesium ammonium phosphate) stones are usually seen in the setting of infection with urease producing bacteria (e.g. *Proteus, Klebsiella, Pseudomonas* and *Enterobacter*), consequenceing in hydrolysis of urea into ammonium and increase in the urinal pH. They can grow very large and form a cast of the renal pelvis and calices consequenceing in so-calledstaghorn calculi. The struvite accounts for approximately 70% of these calculi, and is usually mixed with calcium phosphate thus rendering them opaque. Uric acid and cystine are also found as minor components. Struvite stones are usually large (staghorn calculi) and consequence from infection. These stones need to be treated surgically and the entire stone removed, including small fragments, as otherwise these residual fragments act as a reservoir for infection and recurrent stone formation.

1.6. Uric acid

Hyperuricosuria is not always associated with hyperuricoaemia, and is seen in a variety of settings, although in most instances uric acid stones occur in patients with no identifiable underlying aetiology. Uric acid crystals form and remain insoluble at acidic urinal pH below 5.

Fig. No: 3: Location of Kidney Stone

1.7. Cystine stones:

Cystine stones are also formed in acidic urine, and are seen in patients with congenital cystinuria.Cystine stones may be difficult to treat and are difficult to shatter with ESWL. Hydration and alkalinisation are usually first line therapy

Drug-Induced Nephrolithiasis

1.8. Ephedrine Calculi:-

Ephedrine and its metabolites (norephedrine, pseudoephedrine, and norpseudoephedrine) are sympathomimetic agents that have been used for the treatment

of enuresis, myasthenia gravis, narcolepsy, and rhinorrhea (Powell, Hsu, Turk, &Hruska, 1998). In addition to numerous side effects, ephedrine and its derivatives have been associated with the production of urinal stones (Blau, 1998). The diagnosis of these calculi is similar to that of other radiolucent calculi. Twenty-four hour urine metabolic analyses can aid in identifying ephedrine or its respective metabolites.

1.9. Guaifenesin Calculi:-

Guaifenesin is a widely used expectorant that has been recently associated with nephrolithiasis. Guaifenesin calculi are radiolucent and present in patients who ingest this medication in excess. Twenty-four hour urine metabolic analysis can aid in the identification of guaifenesin or b-2-methoxyphenoxy-lactic acid.

1.10. Indinavir Calculi:-

Indinavirsulfate (Crixivan) is currently one of the most frequently used protease inhibitors used aligned with human immunodeficiency virus, the virus that causes AIDS. The incidence of calculi in patients taking indinavir ranges from 3% to 20% (Schwartz, Schenkman, Armenakas, &Stoller, 1999). Indinavir calculi are radiolucent when they are pure, and are radiopaque when they contain calcium.

1.11. Xanthine Calculi:-

These stones occur due to a rare hereditary condition with xanthine oxidase deficiency.The deficiency in this enzyme consequences in lessened levels of serum and urinal uric acid. Acidic urine causes crystal precipitation, consequenceing in stone

formation (Bernier, 2005). These stones are also seen in patients treated with iatrogenic inhibition of xanthine oxidase with xanthine oxidase inhibitors for hyperuricosuria such as allopurinol.

1.12. Causes of kidney stones

- Age
- Gender
- Diet
- Family history
- Urinal infections and blockage of the urinal tract
- Kidney diseases, such as cystic kidney disease
- Medicinal condition like gout
- Excess vitamin d intake
- Metabolic disorders, such as hyperparathyroidism.
- Certain medications such as diuretics,calcium based antacids and Inherited disease such as cystinuria, hyperoxaluria, hypercalciuria or hyperuricosuria.

1.13. Kidney **stone s diagnosis**

In order to diagnose a patient with kidney stones, doctors will typically:

- Gather a complete medical history
- Ask about the patient's occupation
- Ask about the patient's food habits
- Order laboratory tests, which include urine and blood tests

1.14. Laboratory tests:

- X-rays
- Ultrasound (sonogram)
- CT (computed tomography) scan
- Intravenous pyelogram IVP)

1.15. Treatments for kidneystone

- Most of the kidney stones pass through the urinal system with plenty of water.
- Extracorporeal shock wave lithotripsy
- Ureteroscopy

Larger stones may be treated with:

- Extra corporeal Shock Wave lithotripsy(ESWL)
- Percutaneous nephrostomynt
- Tunnel surgery

Medical Management:

Effective kidney stone prevention is dependent on the stone type and identification of risk factors for stone formation. An individualized treatment plan incorporating dietary changes, supplements, and medications can be developed to help prevent the formation of new stones. Certain conservative recommendations should be made for all patients regardless of the underlying etiology of their stone disease. Patients should be instructed to increase their fluid intake in order to maintain a urine output of

atleast 2,000 ml/day. Patients should also limit their dietary oxalate and sodium intake, thereby decreasing the urinal excretion of oxalate and calcium. A restriction of animal proteins is encouraged for patients with "purine gluttony" and hyperuricosuria (Sastry J.L.N.2004).

1.16. Chemical composition of stones:

There are several types of renal stones that differ in composition and pathogenesis. The most common type of kidney stone is composed of calcium oxalate and is caused by metabolic disorders that are often treatable.

1.17. Calcium stones

Most stones contain calcium combined with oxalate and phosphate or occasionally uric acid. All calcium stones are radio-opaque, and calcium oxalate and calcium phosphate stones are black, grey, or white and small dense and sharply circumscribed on radiographs

1.18. Hypercalciuria

Hypercalciuria or hypercalcinuria is the condition of elevated calcium in the urine. Chronic hypercalcinuria may lead to impairment of renal function nephrocalcinosis, and renal insufficiency.

1.19. Hypocitrauria

It's also associated with renal litho genesis. Citrate acts in the tubular lumen combining with calcium to form a non-dissociable but soluble complex. Hypocitraturia could consequence from a cause of intracellular acidosis such as nephritic failure potassium deficiency, distal renal tubular acidosis, chronic diarrhea state, and drugs such as acetazolamide.

Some studies suggest people who take supplemental calcium have a higher risk of developing kidney stones, and these findings have been used as the basis for setting the

recommended daily intake for calcium in adults. In the Womens Health Initiative, postmenopausal women who consumed 1000 mg of auxiliary calcium and 400 units of vitamin D per day in seven years had a 17% higher risk of developing kidney stones than subjects taking a placebo.

1.20. Uric acid stone

Uric acid stones are smooth, round, yellow orange and nearly radio graphically transparent unless mixed with calcium crystals or struvite. Diets high in purines, especially those containing meats and fish, consequence in hyperuricosuria, and in combination with low urine volume and low urinal p^H can exacerbate uric acid stone formation.

1.21. Struvite or phosphate stones

Struvite is a crystalline substance composed of magnesium ammonium phosphate. Radiographs depict Struvite stones as large, gnarled, and laminated. They are associated with substantial morbidity infection. Signs of Struvite stones include urinal p^H greater than 7, stag horn calculi, and urease that grow bacteria on culture.

1.23. Cystine stone

Formation of cystine stone is the only clinical expression of cystinuria an autosomal recessive intestinal and renal tubular disorders of four amino acids. Cystine, Arginine, Lysine, Ornithine.

People who are homozygous for Cystinuria excrete more than 600mg per day of insoluble cystine. The stones are greenish –yellow flecked with shiny crystals and are moderately radio –opaque rounded appearance.

1.24. Protease –related stone

This the newest type of stone described. The increasing incidences of HIV-positive patients have led to widespread use of the protease inhibitor Indinavir sulphate. Although the drug is generally well tolerated, it can be associated with urolithiasis 4-12% of patients. It thus may coexist or from a nidus for indinavir stones vice versa.

1.25. Prevention of stone

The first step in preventing kidney stones is to understand what is causing the stones to form. The health care provider may ask the person to try to catch the kidney stone as it passes, so it can be sent to a lab for analysis. Stones that are retrieved surgically can also be sent to a lab for analysis.Kidney stones may be prevented through changes in eating, diet, and nutrition and medications.

1.26. Diet and Nutrition

People can help prevent kidney stones by making changes in their fluid intake. Depending on the type of kidney stone a person has, changes in the amounts of sodium, animal protein, calcium, and oxalate consumed can also help. Drinking enough fluids each day is the best way to help prevent most types of kidney stones. Health care providers recommend that a person drink 2 to 3 litters of fluid a day. People with cystine stones may need to drink even more. Though water is best, other fluids may also help prevent kidney stones, such as citrus drinks.

The following recommendations based on the specific type of kidney stones,

Calcium Oxalate Stones

- Reduction of sodium intake
- Reduction of animal protein, such as meat, eggs, and fish
- Getting enough calcium from food or taking calcium supplements with food
- Avoiding foods high in oxalate, such as spinach, rhubarb, nuts, and wheat bran

Calcium Phosphate Stones

- Reduction of sodium intake
- Reduction of animal protein
- Getting enough calcium from food or taking calcium supplements with food

Uric Acid Stones

- Limiting animal protein

Medications

The health care provider may prescribe certain medications to help prevent kidney stones based upon the type of stone formed or conditions that make a person more prone to form stones:

Hyperuricosuria	: Allopurinol (Zyloprim), which decreases uricacid in the blood and urine
Hypercalciuria	: Diuretics, such as hydrochlorothiazide
Hyperoxaluria	: Potassium citrate to raise the citrate and pH of urine
Uric acid stones	: Allopurinol and potassium citrate
Cystine stones	: Mercaptopropionyl glycine, which decreases cystine in the Urine and potassium citrate
Struvite stones	: Antibiotics, which are bacteria-fighting medications, needed to treat infections, or acetohydroxamic acid with longterm antibiotic medications to prevent infection

1.27. Herbal drugs used in Urolithiasis

Herbs have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend aligned with attack from predators such as insects, fungi and herbivorous animals.Many of these phytochemical have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human disease.Pharmacologists, microbiologists, botanistsand natural products chemists are developing phytochemicals for the treatment of an assortment of diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. Numbers of medicinal plants depict antiurolithiatic activity and play a vital role in the kidney stones treatment.

Table 1. List of plants used antiurolithiatic activity

Botanical Name	Family	Parts used	Extract used	Method of inducing urolithiasis
Holarrhenaantidysenterica	Apocynaceae	Stem	Aqueous - Ethanolic Extract	Ethylene glycol
Pergulariadaemia	Asclepediaceae	Whole plant	Alcoholic extract	Ethylene glycol
Asparagus racemosus	Liliaceae	Roots	Ethanolic extract	Ethylene glycol and ammonium chloride
Hordeumvulgare	Poaceae	Seeds	Ethanolic extract	Ethylene glycol
Mimusopselengi	Sapotaceae	Bark	Petroleum, chloroform, and alcohol	Ethylene glycol
Pinuseldaricamedw	Pinaceae	Fruit	Aqueous Extract	Ethylene glycol
Buteamanosperma	Fabaceae	Stem bark	Ethanolic extract	Ethylene glycol

Crataeva magna lour	Capparaceae	Bark	Ethanolic extract	Lactose and ethylene glycol&ammonium chloride and ethylene glycol
Coleus aromaticusBenth	Lamiaceae	Leaves	Water extract	Sodium oxalate
BenincasaHispida	Cucurbitaceae	Seed	Ethanolic Extract	Ethylene glycol
PashanabhedadiGhrita	Saxifragaceae	Root	Ethanolic Extract	ammonium oxalate rich diet and gentamicin injection
AervaLanata	Amaranthaceae	Flower	Aqueous extract	Ethylene glycol
Raphanussativus	Brassicaceae	Bark	Aqueous extract	Zinc disc
Lantana camara	Verbenaceae	Flowering plant	Ethanolic extract	Zinc disc

1.28. Investigational design

A number of model using for study of antiurolithiatic activity. An appropriate investigational urolithiasis model is of importance for studying the pathogenesis of urinal tract stone, evaluating the relative importance of an assortment of lithogenic factors and assessing the efficacy of different drugs in preventing stone formation.

The four models used for inducing lithiasis in rats, they are

1.29. Methods to evaluate Antiurolithiatic activity:

Chemical induced lithiasis

- Sulfadiazine induced urolithiasis
- Sodium glycolate induced urolithiasis
- Ethylene glycol induced urolithiasis

Foreign body insertion method

- Calcium oxalate crystal implantation method
- Zinc bead implantation method

***Invitro* model**

Diet induced lithiasis

2. LITERATURE REVIEW

Neogi, et al. **(1970)** perceived and documented Achyranthine a water soluble alkaloid which possess pharmacological actions like dilation of the blood vessels, lowering of the blood pressure, depression of the heart and increase the rate and amplitude of respiration.

Pawar et al. **(1991)** explained the leaf optical characteristics of *achyranthus-aspera* l growing along agra-bombay road, indore (MP), Reduction in light reflectance and transmittance of visible light from adaxial and abaxial surfaces of dusted and undusted leaves of Glochidion velutinum due to deposit of pollutants was observed. Abaxial surface reflected more light than adaxial. Variation in reflectance & transmittance was found to be related to the amount of surface deposition of pollutants.

Rameshwar et al., **(1993)** revealed three oleonolic acid glycosides from the seeds of *Achyranthes aspera* which were identified as α-L rhamnopyranosyl-(1,4)-(β-Dglucopyranosyluronic acid)-(1,3)-oleanolic acid, 28–O–β–D-glucopyranoside and α-Lrhamnopyranosyl-(1,4)-(β-D-glucopyranosyluronicacid)-(1,3)-oleanolicacid, 28–O-β-Dglucopyranosyl-(1,4)-β-D-glucopyranoside..

Misra, et al., **(1993)** perceived and documented certain long chain compounds from the shoots like 27-cyclohexylheptacosan-7-ol and 16-hydroxy-26-methylheptacosan-2-one.

Talakal T.S, et al., **(1996)** In vitro screening of some indigenous plants aligned with Trypanosoma evansi, Aqueous extracts of 9 indigenous plant materials were screened in vitro for their activity aligned with Trypanosoma evansi at concentration of 5, 50, 500 and 1000 mu g/ml. The extracts of leaves of Glochidion velutinum , Caesalpinia bonducella and Dhatura alba did not depict

activity at any concentration tested. The extracts of other plants,viz. Azadirachta indica leaves, Cassia occidentalis leaves, Cyperus rotundus rhizome, Hydrocotyle asiatica leaves and Streblus asper leaves, exhibited moderate trypanocidal activity at different concentrations tested. However, the extract of Nyctanthes arbor-tristis at a concentration of 1000 mu g/ml was highly effective.

Misra, ***et al.*** **(1996)** isolated an assortment of compounds like tetracontanol-2 ($C_{40}H_{82}O$, melting point 76-77°C), 4-methoxyheptatriacont-1-en-10-ol ($C_{38}H_{76}O$) and β-sitosterol .

Kunert, et al. **(2000)** perceived and documented three bisdesmosidic saponins (I-III), 20-hydroxyecdysone, and quercetin-3-O-β-D-galactoside, were isolated from the methanol extract of the aerial parts of *Achyranthes aspera*.

Schmid, et al., **(2000)** perceived and documented two new bisdesmosidic triterpenoid saponins were isolated, besides the three known saponins from the Ethanolic extract of the aerial parts of*Achyranthes aspera*. Their structures were elucidated as β-D-glucopyranosyl3β-[O-α-L-rhamnopyranosyl-(1,3)-O-β-D-

Nasare P, et al., **(2000)** Therapeutic efficacy of an indigenous drug formulation in investigational hepatopathy and nephropathy in goats, Adult goats (19) of either sex were used to observe the efficacy of an indigenous drug formulation in hepatopathy and nephropathy by taking oxytetracycline-induced toxicity model. Animals were divided into groups 1, 2 and 3. Groups 1 and 2 were divided into subgroups A and B, consisting of 4 animals each. Subgroups A and B received oxytetracycline (OTC) 25 mg/kg b.wt and 40 mg/kg bwt respectively. Group 3 consisted of 3 animals and was maintained as healthy control. In addition to OTC, groups 1 and 2B received an indigenous drug formulation @ 10 g orally bid for 10 days. It consisted of Terminalia arjuna, Andrographis paniculata, Eclipta erecta, Trianthema decandra, Piper chaba, Saxifruga linguilata,

Glochidion velutinum, Onosma bracteanum, Tinospora cardifolia. The toxicity of OTC and efficacy of indigenous drug formulation was assessed by haematological evaluations and biochemical evaluations consisting of liver function test and kidney function test. Indigenous drug was observed to be an effective adjuvant therapy for OTC-induced hepatopathy and nephropathy.

Gokhale, et al., (**2001)** perceived and documented the ethanolic extracts of the *Achyranthes aspera* at the doses of 50, 100 and 200 mg/kg were screened for their effect on acute and chronic inflammation induced in mice and rats using carrageenan and Freund's complete adjuvant model. *A. aspera* inhibited these inflammatory responses at doses of 100-200 mg/kg.

Srivastava S, et al., (**2002**)A new oleanolic acid saponin from Glochidion velutinum , Butanol extract of Glochidion velutinum inflorescence afforded a new compound which was characterized as beta-D-fucopyranosyl-(1-->4)-(beta-D-glucopyronosyluronic acid)-(1-->3)-oleanolic acid.

Gokhale, et al., (**2002)** perceived and documented the ethanolic extracts of the *Achyranthes aspera* at the doses of 50, 100 and 200 mg/kg were screened for their effect on acute and chronic inflammation induced in mice and rats using carrageenan and Freund's complete adjuvant model. *A. aspera* inhibited these inflammatory responses at doses of 100-200 mg/kg.

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Thilagavathi *G, et al.,* (**2005)** Development of ecofriendly antimicrobial textile finishes using herbs, An assortment of herbal species were screened for

their antimicrobial activities by employing preliminary (qualitative) antimicrobial tests. Ethanolic extraction procedure was followed for extracting the active Substances from herbs. Antimicrobial efficacy was assayed by (agar diffusion and parallel streak) method and Hohenstein modified challenge test. The neem leaves (Azadirachta indica), prickly chaff flower (Glochidion velutinum), tulsi leaves (Ocimum basilicum) and pomegranate rind (Punica granatum) were found to exhibit antimicrobial activity aligned with the strains of Staphylococcus aureus and E. coli. Neem ranked first followed by pomegranate and prickly chaff flower. Despite the negative consequences Of tulsi in the qualitative tests, it depicted 73% bacterial reduction in the quantitative challenge test. The treated fabric samples exhibited resistance to degradability as tested by digging soil test

Laddha, *et al.* (**2005**) perceived and documented extraction, isolation and purification of 20- hydroxyecdysone from *Achyranthes aspera* and its characterization by DSC, UV, IR, CD, $_{1}$H and $_{13}$C NMR, MS and quantification by HPLC.

Ravindra, et al., (**2006**) Executed the work with the Effect of *Moringa oleifera* Lam.root-wood on ethylene glycol induced urolithiasis in rats.

Naveed, et al., (**2007**) Contribution of cultivated crops, vegetables, weeds and ornamental plants in harboring of Bemisia tabaci (Homoptera : Aleyrodidae) and associated parasitoids (Hymenoptera : Aphelinidae) in cotton agroecosystem in Pakistan, The population dynamics of Bemisia tabaci and its parasitoids was studied on Gossypium hirsutum, Cucumis melo, Helianthus annus, Glycine max, Solanum melangena, Cucurbita pepo melopopo, Bauhinia pupurea, Morus alba, Albizzia lebbek, Lantana camara, Glochidion velutinum , and Convolvulus arvensis in cotton growing areas of Punjab, Pakistan during 2004 and 2005. Whitefly infested leaves having maximum number of second to third instar were collected and kept in glass petri dishes with lid on at 28 +/- 2 degrees C and 65 +/- % RH. Mean population of whitefly adults that emerged per 200 cm(2) leaf area

per sampling period recorded was maximum on G. hirsutum (43.2), followed by C. melo (31.5), L. camara (23.0), H. annus (20.5), G. max (19.3), C. pepo melopopo (18.1), S. melangena (16.9), A. aspera (11.2), C. arvensis (9.2), B. pupurea (5.4), M. alba (5.3) and A. lebbek (5.0). Percentage parasitism was higher on G. hirsutum (44.3%), followed by C. melo (38.9%), A. aspera (38.3%), L. camara (38.1%), A. lebbek (35.3%), G. max (33.5%), C. arvensis (33.0%), M. alba (31.1%), B. pupurea (27.0%), S. melangena (24.8%), C. pepo melopopo (16.1%) and H. annus (15.2%). Overall the population of whitefly remained low during winter (November-February) and high during summer (May-August) whereas, the percentage parasitism was higher during June-September and lower during December-February. The study revealed that the availability of parasitoids could be enhanced by planting

3. PLANT PROFILE

MORPHOLOGY OF *GLOCHIDION VELUTINUM*

Fig 4. Habit of *Glochidion velutinum*

Fig 5. Entire plant

Description

Kingdom- Plantae

Family-Phyllanthacea

Genus-Glochidion

Species-*Glochidion velutinum*

Vernacular names

English-Velvetymelon feather foil

Malayalam-Cthakkatanv

Tamil-Panickaavu

Geographical source

Glochidion velutinum Linn.locally is one of the most important customaryly used antifertility plants in the indigenous health care delivery system of Ethiopia.Easily found anywhere in India on road sides or on the edges of field and waste places as a weed throughout up to an altitude of 2100 m and also in South Andaman Islands Some other places in the world also This plant is widespread in the world as a weed, in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America..

Habit and Habitat:

The plant is distributed throughout India up to an altitude of 3000ft. Erect or ascending herbs or shrubs. It is growing in rainyseason.It is an erect, ligneous, 0.8-1 m height with stiff branches, cerate or absolutely quadrangular plant.

Therapeutic uses

- Antidiabetic
- Antioxidant
- Anticancer
- Antiinflammatory
- Antimicrobial
- Antiurolithic

Phytochemical constituents

- Alkaloids
- Tannins
- Saponins
- Proteins and free aminoacids
- Flavanoids
- Carbohydrates

4. AIM AND OBJECTIVE

Most of the plants are used in the alternative system of medicine but they are not systematically standardised. According to WHO guideline all the customary drugs should be standardised before going for the formulation. One of the WHO assembly resolutions emphasized the need to ensure quality control of medicinal plants products using modern techniques and establishment of required standards of quality for herbal medicines. So the objective of the project is to evaluate the pharmacognostical and phytochemical characters' of the selected plants by advanced techniques.

Exploiting the leaves of *glochidion velutinum*

- ✓ Phytochemical
- ✓ Pharmacological aspects with special emphasis on anti urolithiatic activity.

5. PLAN OF THE WORK

The following are the plan of work

- To perform the literature review
- To perform the collection and extraction of leaves of *glochidion velutinum*
- To perform the Phytochemical tests
- To perform the Antiurolithic activity
- To perform the Antioxidant activity

6. MATERIALS AND METHODS

Authentication of Plant Materials

The leaves of *glochidion velutinum* available locally were collected in and around collected from RR district in Telangana. The voucher specimens had been submitted and preserved in herbarium for future reference.

Physico Chemical Parameters

The three plant leaves were subjected for the physicochemical parameters like Ash values, Extractive values, Total fiber contents, Moister content .The consequences obtained in that was perceived and documented.

Processing of Plant material

The plant materials were collected and shade dried at room temperature and was subjected to size reduction to get course powder of desired particle size. Then powdered and passed through mesh size 40 and stored in air tight containers.These powdered materials were subjected to successive extraction. Each (1kg) powdered drugs were extracted with methanol and water separately by cold maceration method for 7 days. Then the extracts were filtered and solvents were evaporated under abridged pressure in a rotary evaporator to get the dry extract. The yield of the dry extracts were calculated and stored in desiccators and used for further experiments.

Preliminary phytochemical analysis

The Ethanolic and aqueous extracts of the plant materials were separately prepared and subjected to chemical tests for the identification of its chemical constituents. Chemical tests were carried out on the aqueous and methanol extracts and on the powdered specimens using standard procedures to identify the constituents

1.Test for alkaloids

Mayer's Test (Potassium Mercuric Iodide)

A fraction of the extract was treated with Mayer's reagent and observed for the formation ofa cream coloured precipitate.

Dragondroff's Test (Potassium Bismuth Iodide)

A fraction of the extract was treated with Dragondroff's reagent and observed for the formation of a reddish coloured precipitate.

Wagner's Test (Iodine in Potassium Iodide)

A fraction of the extract was treated with Wagner's reagent and observed for the formation of a reddish brown precipitate.

Hager's Test (Picric acid Test)

A fraction of the extract was treated with Hager's reagent and observed for the formation of a yellow coloured precipitate.

2.Test for carbohydrates

Molisch's Test

A fraction of the extract was separately treated with a solution of β-naphthol and few drops of concentrated sulphuric acid were added slowly through the side of the test tube. It was observed for the formation of a violet ring between the junctions, which indicates the presence of carbohydrates.

Fehling's Test

A little of the extract was treated with Fehling's solution A and B and heated on a water bath for few minutes. It was observed for the formation of a red precipitate of cuprous oxide.

Barfoed's Test

A small portion of the extract was treated with Barfoed's reagent and observed for the formation of a red precipitate.

3.Test for proteins

Millon's Test

To the extract, a little water and Millon's reagent was added. Appearance of red colour depicted the presence of proteins.

Ninhydrin Test

To the extract, a little of Ninhydrin reagent was added. Appearance of purple colour depicted the presence of proteins.

Biuret Test

To the extract, a small amount of sodium hydroxide and copper sulphate solution was added. Appearance of violet colour indicated the presence of proteins.

Xanthoprotein Test

To the extract, equivalent quantity of concentrated nitric acid was added and boiled. It was made alkaline with sodium hydroxide solution. Yellow colour changing to deep yellow or orange indicates the presence of protein.

4.Test for tannins and phenolic compounds

Ferric Chloride Test

To a small quantity of the extract, few drops of neutral ferric chloride solution was added and observed for formation of brownish colour.

Lead Acetate Test

To the extract, 10% lead acetate solution was added and observed for formation of white precipitate.

Gelatin Solution Test

To the extract, 1% solution of gelatin containing sodium chloride solution was added and observed for the formation of white precipitate.

5.Test for phytosterols and terpinoids

Salkovaski Test

A small quantity of chloroform extract was treated with 5ml of concentrated sulphuric acid. The solution changed colour from yellow to red at the junction indicating

the presence of terpenoids.

Libermann – Burchard Test

A small quantity of chloroform extract was treated with 0.2ml concentrated sulphuric acid and 4ml acetic anhydride. The solution turned pink in colour and finally became purple to Red colour.

6. Test for glycosides

Borntragers Test

The powdered drug extract boiled with dilute sulphuric acid. Filtered hot and to the cooled filtrate, add 5ml of ether and shake well. Separate the organic layer and add equal volume of dilute ammonia solution. Shaked well, no change in the ammoniacal layer.

Modified Borntragers Test

Shaked the extract with 5ml of ferric chloride solution mixed with 2.5ml of hydrochloric acid. Heated it in a water bath for 10 minutes. Filtered and extracted the filtrate with 5ml of carbon tetrachloride. Separated the organic layer and treat with 5ml dilute ammonia solution. No change in the ammoniacal layer.

Keller – Killiani Test

Boiled the extract with 70% alcohol for 3 minutes. Filtered and to the filtrate added 5ml of water and 0.5ml of strong solution of lead acetate. Shaked well and filter. The clear filtrate is treated with equal volume of chloroform and chloroform layer is evaporated. The residue is dissolved in 3ml of glacial acetic acid and to this 2 drops of ferric chloride solution is added. The contents are transferred to a test tube containing 2ml of concentrated sulphuric acid. There was no colour reaction observed.

Legal's Test

Dissolved the extract in pyridine, added 2ml sodium nitroprusside solution and made alkaline with sodium hydroxide solution. No colour change.

Baljet's Test

The extracted is treated with sodium picrate reagent. No colour change.

7.Test for Flavanoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixturewas filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Shinoda test:

To the ethanolic extract added and few drops of concentrated HCl. To this add 0.5 gm magnesium turnings were added. Pink colour formed which indicated the presence of flavonoids.

Lead acetate test: To the ethanolic extract lead solution was added. Formation of Yellow Precipitate depicted the presence of Flavonoids.

8.Test for Saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

9.Test for Steriods:

Two ml of acetic anhydride was added to 0.5 gethanolic extract of each sample with 2 ml H_2S0_4. The colourchanged from violet to blue or green in some samples indicatingthe presence of steroids.

10.Test for Terpenoids:

Five ml of each extract wasmixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to depict positive consequence for the presence of terpenoids.

Table 2. Estimation of Extractive values and Ash values

S.No.	Parameters	GV (% w/w)
1	**Extractive Values**	
a.	Petroleum ether	16.23
b.	n-hexane	3.3
c.	Chloroform	18.63
d.	Methanol	20
e.	Water	13.63
2	**Ash Values**	
a.	Total Ash	6.35
b.	Acid insoluble Ash	2.54
c.	Water soluble Ash	1.43
d.	Sulphated Ash	2.13
3	Loss on Drying	0.89
4	Crude fibre content	10.2

Experimental Design

Animal selection

Healthy Inbred Albino rats of *Wistar* strain, of male, aged around 2 to 3 months and weighing 150-200 g were selected for the antiurolithiatic activity used. The animals were acclimatized to standard laboratory conditions (temperature:25±2 ◦C) and maintained on 12-h light-dark cycle , relative humidity of 45-55%, and maintained on

12–hour light: 12–hour dark cycle in animal house. They were provided with regular rat chow (Lipton India Ltd., Mumbai,India) and drinking water ad libitum. The animal care and investigational protocols were in accordance with Institutional Animal Ethical Committee (IAEC).

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD50) was taken as an effective dose. The acute oral toxicity study was carried out as per the OECD guidelines usinFor acute toxicity studies, Wistar albino mice of either sex weighing between 25 and 30 g were selected and employing the up and down method prior to evaluating all the extracts for antiurolithiatic activity. One-tenth of the median lethal dose (LD50) was taken as an effective dose.

Antiurolithiatic Activity Study

Chemicals

All the chemicals and reagents were purchased from Merck, Mumbai, India. Solventsand all the reagents used were of analyticalgrade. The creatinine kit purchased from (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kits from (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid level.

Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats. Animals were divided into nine groups containing six animals in each.

Treatment protocol

The grouped animals received the treatment as follows

Group I – Received normal diet and served as controls.

Group II - *Lithiatic control:* The animals were given normal diet and 1% Ethylene glycol in drinking water, for 28 days.

Group III - Received 1% ethylene glycol in drinking water and then treated with Ethanolic extract of GV at a dose of 200mg/kg orally, for 28 days.

Group IV - Received 1% Ethylene glycol in drinking water and then treated with Aqueous extracts of GV at a dose of 200mg/kg orally, for 28 days.

All extracts were given once daily by oral route.

Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine samples were collected on 14^{th}, and 28^{th} day of calculi induction treatment. The volume and calcium content of urine were measured. Calcium in urine was estimated using kit by COBAS MIRA PLUS auto analyzer. Urine was analyzed for oxalate,magnesium, phosphate, uric acid, citrate and total protein

Serum analysis

The blood was collected from the retro-orbital sinus under anaesthetic condition and serum was separated by centrifugation at 10,000rpm for 10 min and analyzed for creatinine and uric acid. The creatinine kit (Reckon Diagnostics Pvt. Ltd., India)and uric acid diagnostic kit (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid levels respectively.

Kidney histopathology

The abdomen was cut open to remove both kidneys from each animal. Isolated Kidneys were cleaned off extraneous tissue and rinsed in ice-cold physiological

saline..The right kidney was fixed in 10% neutral buffered formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm and Stained with H and E (Haematoxylin and Eosin) for histopathological examination.The slides were examined under light microscope to study microscopic network of the kidney and calcium oxalate sediments.

Enzyme assays

A portion of kidney was taken from all the groups,and a 30% w/v homogenate was prepared in 0.9% buffererd KCL (pH 7.4)for the estimation of glutathione(GSH), Super oxide dismutase (SOD), catalase (CAT) and malondialdehyde(MDA).

Antioxidant Activity

Renal cellular exposure to oxalate (Ox) and/or CaOx crystals leads to the production of Reactive Oxygen Species (ROS), development of oxidative stress followed by injury and inflammation. Renal injury and inflammation appear to play a noteworthy role in stone formation. An overproduction of ROS and a reduction in cellular antioxidant capacities, due to down-regulated expression of the antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glucose-6 phosphate dehydrogenase) as well as radical scavengers (vitamin E, ascorbic acid, abridged glutathione) leads to the development of Oxidative Stress (OS) (Ying, W. M,2002) .Oxidative stress followed by renal cell injury and inflammation due to lipid peroxidation (Udupa. K.N., Singh R.H.1995). Loss of membrane integrity subsequently facilitates the retention of calcium oxalate crystals and growth of stones in renal tubules .Recent studies have provided evidence that CaOx kidney stone patients malondialdehyde (MDA) in their urine, indicating ROS in kidneys of CaOx stone patients. Urinal excretion of these MDA is considered as a marker of renal epithelial cell injury.

Recent studies evidenced that treatment with anti-oxidants and free radical scavengers abridged CaOx crystal induced renal injuries. Pre-treatment with vitamin E along with mannitol abolished the deposition of CaOx crystals in the kidneys of rats injected with sodium oxalate (A.Helen, K.Krishnakumar 2003). Alanine-induced deposition of CaOx crystals in rat kidneys was blocked by dietary supplementation with

vitamin E plus selenium. These antioxidant therapies restore the activity of antioxidant enzymes and free radical scavengers (Zima, T.S.2001). Therefore, treatments with natural antioxidants and free radical scavengers, seems to possible thereupatic strategy for ameliorating hyperoxaluria induced oxidative stress and renal cell injury in urolithiasis. Herbal medicine or plants are rich source of natural antioxidants, can be used in treatment of hyperoxaluria induced oxidative stress and urolithiasis. Protective effect of herbals in hyperoxaluric oxidative stress and CaOx crystal deposition is due to their potential antioxidant activity depicts reduction in oxalate-induced renal tubular epithelial cell injury in cell culture due to their antioxidant activity.

DPPH radical scavenging activity:

The free radical scavenging activity was measured by (Kumaran A 2007) method, the decrease in absorbance of Ethanolic solution of DPPH. A stock solution of DPPH ($33mgL^{-1}$) was prepared in methanol and 5ml of this stock solution was added to 1ml of the plant extract solutions at different concentrations ($25,50,75,100,150,200,250,2500ug/ml^{-1}$).After30min, absorbance was measured at 517nm and compared with the standard ascorbic acid ($10\text{-}50ugml^{-1}$) pH 7.4.Percentage of DPPH scavenging activity of the plant extracts and the standard was calculated. The percentage extract of inhibition was calculated by the formula [(Ao-A1)/Ao] x100, when Ao is the absorbance of the control & A1 is the absorbance of the extract/standard.

Reducing power determination

Reducing power determination using potassium ferricyanide (Oyaiz*u et al., 1986*). The reducing power of the extracts was determined with different concentrations of extracts/standard (50-250mgm/ml) in methanol were mixed with phosphate buffer (PH 6.6) and incubated with (2.5ml) of potassium ferricyanide solution (1%w/v) at $50^{o}C$ for 20 min. Then 2.5 ml of trichloroacetic acid was added to the mixture and which was then centrifuged for 10 min. The supernatant (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Scavenging of hydrogen peroxide:

Capability of three extracts to forage hydrogen peroxide was determined by ((Ruch.R.J.1984) method .A solution of H_2O_2 (2mol/l) has been prepared in phosphate buffer-PH 7.4. Hydrogen peroxide concentration was determined by spectrometric ally absorbance at 230nm.Extracts were prepared at the concentration of 50-250mgm/ml and added to the H_2O_2 solution (0.6ml). Blank contains phosphate buffer with without H_2O_2. Each concentration a particular blank sample was utilized taking the reading. The % of inhibition effect was calculated from the formula $[(A_0 - A_1)/A_0]$ X 100.Where as $A_1$is the absorbance of extract/standard and A_0 is the absorbance of the standard and.

Assay of nitric oxide scavenging activity:

The nitric oxide scavenging activity of the samples was calculated as per the scheme of (Sreejayan and Rao., 1997). 3ml of Sodium nitropruside in 0.2 M in phosphated saline (pH 7.4) with special strenght of Ethanolic extracts and incubate at room temperature for 2 hours. Same reaction component devoid of the methanol extract but the equivalent amount of methanol served as the control.After the period of incubation, 0.5 ml of reagent Griess was added.The absorbance of the chromophore was measured at 546nm.Quercetin and the same mixture of the reaction without the sample extracts were employed as positive and negative control. The range or degree of radical scavenging activity was measured as

% NO radical scavenging activity = (control OD - sample OD / control OD) × 100.

The analysis was done three times. Sample strength lending 50% inhibition (IC 50) under the assay condition was measured from the pictorals of inhibition percentage aligned with sample concentration.

Statistical analysis

The consequences were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Newmannkeul's multiple range tests and $p < 0.05$ was considered noteworthy.

7. RESULT AND DISCUSSION

7.1 The Phytochemical screening results are as follows

Table 3. Phytochemical screening

S.no	Chemical Test	GV	
		Aqeous	Alcohol
(1)	Alkaloids		
A	Mayer's test	+	+
B	Dragendroff's test	+	+
C	Wagner's test	+	+
D	Hager's test	+	+
(2)	Carbohydrates		
A	Molisch's test	+	+
B	Fehling's test	+	+
C	Barfoed's test	+	+
(3)	Proteins and Free Amino acids		
A	Ninhydrin test	_	-
B	Biuret test	+	+
C	Xantho Protein test	+	+
(4)	Tannins and Phenolic Compounds		
A	Ferric chloride test	+	+
B	Lead acetate test	+	+
C	Gelatin test	+	+
(5)	Phytosterols		
A	Libermann Burchard test	-	+
B	Salkowski test	-	+
(6)	Flavanoids		
A	Shinoda test	+	+
(7)	Saponins	+	+
(8)	Glycosides	-	-
9	Terpenoids	-	-

The phytochemical screening result shows Alkaloids,Carbohydrates,Proteins and aminoacids,Tannins and phenolic compounds, Phytosterols,Flavanoids,Saponins,etc

7.2 Antiurolithiatic Activity:-

In the present study, the urine amount augmented in the treated group's animals than that of the control and it abridged in the untreated lithiatic animals when comparing to the standard and urinal concentration of the an assortment of ions investigated varied drastically, subsequent ethylene glycol treatment in the lithiatic control. The oxalate, calcium, uric acid, creatinine and phosphate excretion were notably increased on day 14^{th}& 28^{th} respectively for GROUP-II following ethylene glycol treatment. Management with Ethanolic and aqueous extracts of *Glochidion velutinum* abridged the excretions notably on 14^{th} day of treatment and supplementary abridged on 28^{th} day, like standard. In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were augmented notably in GROUP-II (Lithiatic control) following ethylene glycol treatment. Treatment with Ethanolic and aqueous extracts of Glochidion velutinum abridged all above mentioned parameters notably. On the contrary, the magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol action**.** After management with Ethanolic and aqueous extracts of *Glochidion velutinum* the magnesium level was restored near to regular and standard levels.

Low urinal magnesium content is a general attribute in stone formers. An alike condition was observed in the (GROUP-II) rats. Management with Ethanolic and aqueous extracts of *Glochidion velut*inum elevated the urinal magnesium level, and consequently, abridged the propensity to crystallize, thereby creating an ambience unfavorable for precipitation. Increased excretion of proteins has been distinguished in hyperoxaluric rats and stone formers.

In this study before estimating the ionic concentration of the urine on 14^{th} and 28^{th}day, the total quantity of urine excreted by the 9 groups were estimated. The treated groups depicted augmented the amount of urine volume. The consequences were expressed in table no1. In the 14^{th} and 28^{th} day after treatment the Urine parameters such as calcium, uric acid, creatinine, oxalate, phosphate and magnesium levels were estimated and documented in the table no. 2 and table no3. Then the serum parameters such as calcium,

uric acid, creatinine, oxalate, phosphate levels were estimated on 28th day of the treatment and that was documented in the table no.4. The kidney was prepared for the estimation of glutathione(GSH)Super oxide dismutase (SOD) catalase (CAT) and malondialdehyde (MDA) that enzyme level was documented in the table no.5.

Table 4. Effect of *Glochidion velutinum* on urinal output in urolithiasis induced rats.

Days	**Group-I**	**Group -II**	**Group-III**	**Group-IV**
0	7.25±0.52	7.30±0.60	7.35±0.66	7.90±0.70
14	7.89±0.60	5.35±1.36	6.45±1.50	6.20±1.20
28	7.56±0.76	4.95±1.60	6.95±1.86	7.75±1.30

Table 5. Effect of *Glochidion velutinum* on urinal Biochemical parameters on the 14th day

Groups	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	70.90±3.76	4.42±0.58	6.15±0.70	3.30±0.62	0.90±0.08	16.30±1.50	33.60±2.26
Group -II	158.40 ±7.30	1.35 ±0.11	22.15 ±1.60	13.60±1.32	1.86±0.24	48.20 ±4.45	78.66±4.26
Group -III	93.12 ±5.78	2.95 ±0.38	12.65 ±0.95	8.40 ±0.86	1.22 ±0.16	24.38 ±2.68	44.82 ±3.32
Group -IV	88.56 ±5.26	3.26±0.45	10.66±0.76	6.05±0.76	1.08 ±0.10	18.10 ±2.50	37.55±3.06

Table 6. Effect of *Glochidion velutinum* on Urinal Biochemical parameters on 28th day

Groups	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	65.96±2.86	4.20±0.52	5.63±0.54	3.22±0.65	0.80±0.08	15.80±1.83	32.90±2.20
Group -II	152.22 ±6.30	0.98 ±0.14	20.15±1.98	12.5 ±1.62	1.56 ±0.14	32.65 ±3.42	73.60 ±4.26
Group -III	86.20 ±4.20	2.50 ±0.36	9.45 ±1.10	5.5 ±0.94	0.92 ±0.09	23.22 ±2.76	43.55 ±3.73
Group -IV	82.66 ±3.55	2.88 ±0.40	8.90 ±0.92	4.20 ±0.85	0.86 ±0.11	19.30 ±2.32	37.80 ±3.15

Table 7. Effect of *Glochidion velutinum* on serum Biochemical parameters on 28day

Groups	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	4.80±0.86	9.40±1.32	3.45±0.40	0.56±0.03	6.6±0.57	12.06±1.43
Group –II	1.38±0.25	18.30±2.34	9.7±1.10	1.01±0.13	12.60±1.61	26.01±3.25
Group -III	3.28±0.46	11.85±1.88	4.55±0.55	0.91±0.09	8.45±0.88	20.10±2.65
Group –IV	3.67±0.52	11.22±1.60	4.10±0.46	0.80±0.07	8.12±0.78	19.85±2.05

GP_1- Normal; **GROUP-II**- Lithiatic Control; **GP_3**- EEGV (200mg/kg); **GP_4**- AEGV(200mg/kg); **GP**- Cystone herbal tablets (100mg/kg)

- Values are expressed in ml/24 h urine sample mean ± SEM
- Values were originate out by by means of ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were notably different from normal control (GP_1) at P< 0.01
- **(b) Values were notably different from Lithiatic control (GROUP-II) at P<0.01

7.3 Histopathological study:

The abdomen was cut open to take away both kidneys from each animal. Secluded Kidneys were made tidy away from extraneous tissue and rinsed in ice-cold physiological saline.The right kidney was prepared and stained for histopathological examination.The slides were examined under light microscope to study microscopic design of the kidney and calcium oxalate deposits. The photo from the histopathological examination gave a clear consequence ,that depicts the reduction in the stone development and the inflammations also abridged when

comparing with the standard drug and the untreated group depicted large quantity of microcrystal deposition and stern dilation of most tubules and mass tubulointerstitial inflammatory infiltration with lesion area .It has been noted in the figure 6,7,8,9

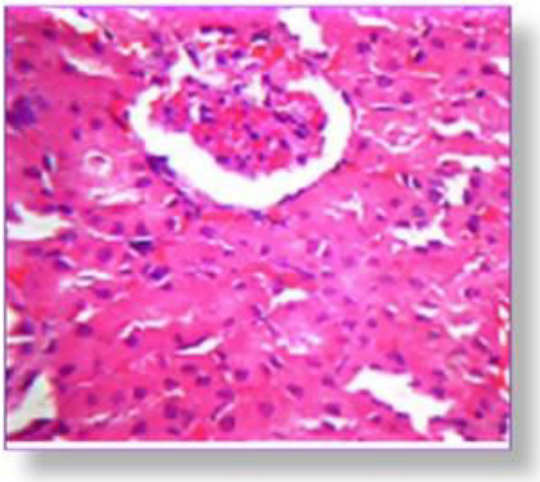

Fig 6. Section of kidney glomerului-I

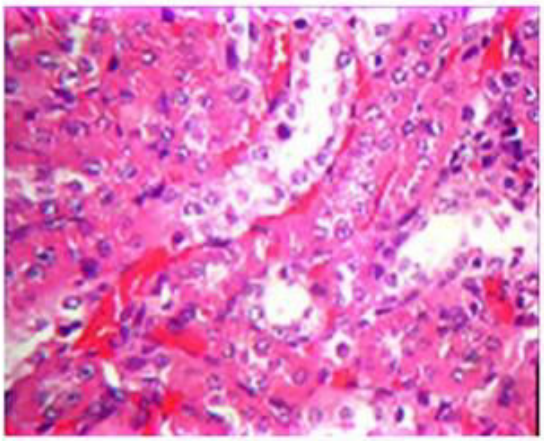

Fig 7. Section of kidney glomerului-II

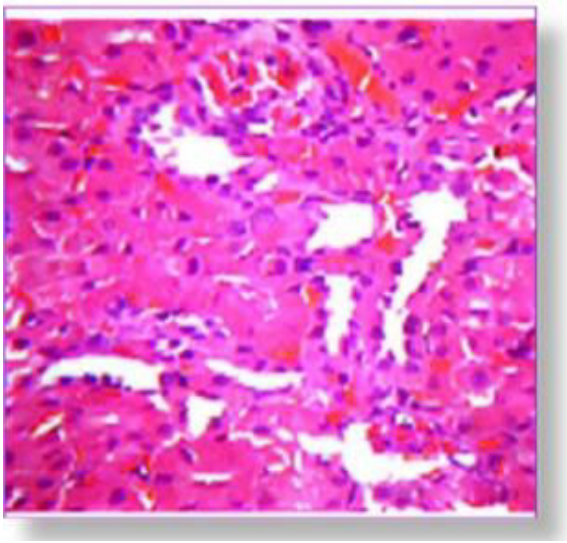

Fig 8. Section of kidney glomerului-III

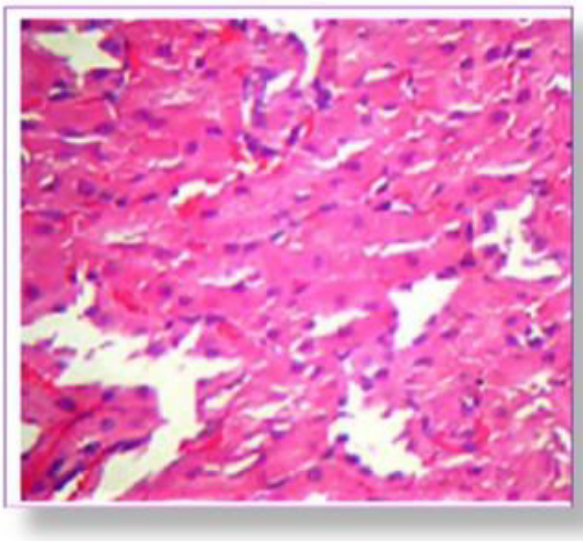

Fig 9. Section of kidney glomerului-IV

In vivo antioxidant activity

For *invivo* antioxidant action EtOH treatment augmented MDA (P<0.01) and lessened GSH (p<0.01) SOD (p<0.01) and CAT (0.01) levels in control animals. Aqueous and ethanolic extracts of Glochidion velutinum at a dose of 200mg/kg produced noteworthy (p<0.001) reduction in MDA and augmented GSH and antioxidant enzyme likes SOD and CAT compared to standard group cystone (table 5).

Table 8. Effect of Aqueous and Ethanolic extracts of *Glochidion velutinum* on antioxidant enzymes in renal tissue

Treatment	Catalase A/protein	SOD B/mg protein	GSH nmoles/mg protein	MDA nmoles/mg protein
Normal control	3.20±0.18	9.20±0.18	3.72±0.16	3.88±0.28
Ethylene glycol	0.98±0.02	3.68±0.07	0.52±0.06	10.05±0.46
Cystone	2.80±0.16	7.05±0.11	2.68±0.14	4.20±0.30
AEGV 200mg/kg	2.32±0.09	6.55±0.10	2.12±0.10	5.68±0.35
EEGV 200mg/kg	2.28±0.08	6.60±0.12	2.20±0.11	5.76±0.45

- Values are expressed as Mean± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a)values were notably different from normal control (GP_1)at P< 0.01
- **(b) values were notably different from Lithiatic control (GROUP-II)at P<0.01

7.4 Antioxidant activity

All these three plants are having antioxidant action. It was evaluated by five technique. In the Hydrogen peroxide scavenging action method the IC50 values are calculated and comparing with the standard Ascorbic acid, it depicts 140 µg of *Glochidion velutinum* is corresponding to that of 12.5 µg of ascorbic acid. The anti-haemolytic activity method, IC50

values are calculated and compared with the standard Quercetine .The consequence depicts 211.29µg of *Glochidion velutinum* is corresponding to 57.06 µg of Quercetine. Flavonoids are phenolic components, abundant in several plants, which inhibit lipid per oxidation and lipoxygenases *invitro* and in presence of Fe^{3+}.In the lessening power action method the IC50 values are calculated and distinction with the paradigm Epicatechine. The outcome depicts 114 µg of *Glochidion velutinum* is equivalent to 12.5 µg of Epicatechine action.

NO is an imperative component intermediary generated by endothelial structures, macrophages, neuronals and troubled in the directive of an assortment of physiological processes. Excess concentration of NO is implicated in the cytotoxic trait pragmatic in an assortment of disorders like AIDS, cancer, Alzheimer's and arthritis. Oxygen reacts with the surplus NO to manufacture nitrite and peroxy nitrite anions, which act as free radicals.[23] From consequences of Nitric oxide technique, it proved that the aqueous leaf extract of *AC* had effectual anti oxidant action.

These extract fight with oxygen to act in retort with NO and accordingly inhibit the generation of the nitrite and peroxy nitrite anions. In NO scavenging action technique the IC50 values are calculated and compared with the standard Quercetine IC50 value. It depicts 294.16 µg of *Glochidion velutinum* is corresponding to 25 µg of Quercetine. Free radical scavenging action of the aqueous leaf extract of *AC* is concentration dependent, as the concentration of the test compounds raised, the radical scavenging activity increases. In the DPPH scavenging action method the IC50 values are calculated and it lends a good consequence of 384µg of *Glochidion velutinum* is equivalent to 24µg of Ascorbic acid.

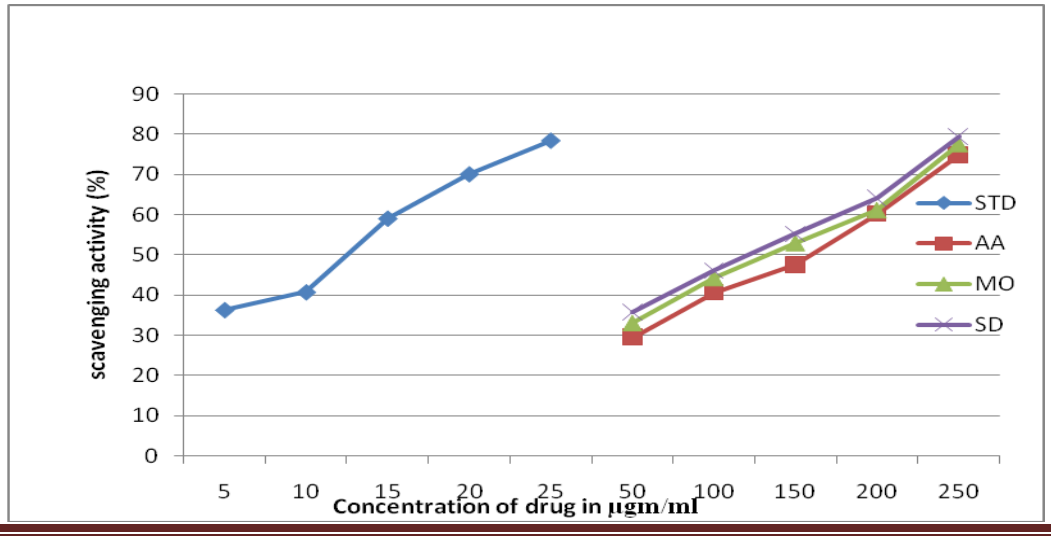

Fig 10. Hydrogen peroxide method

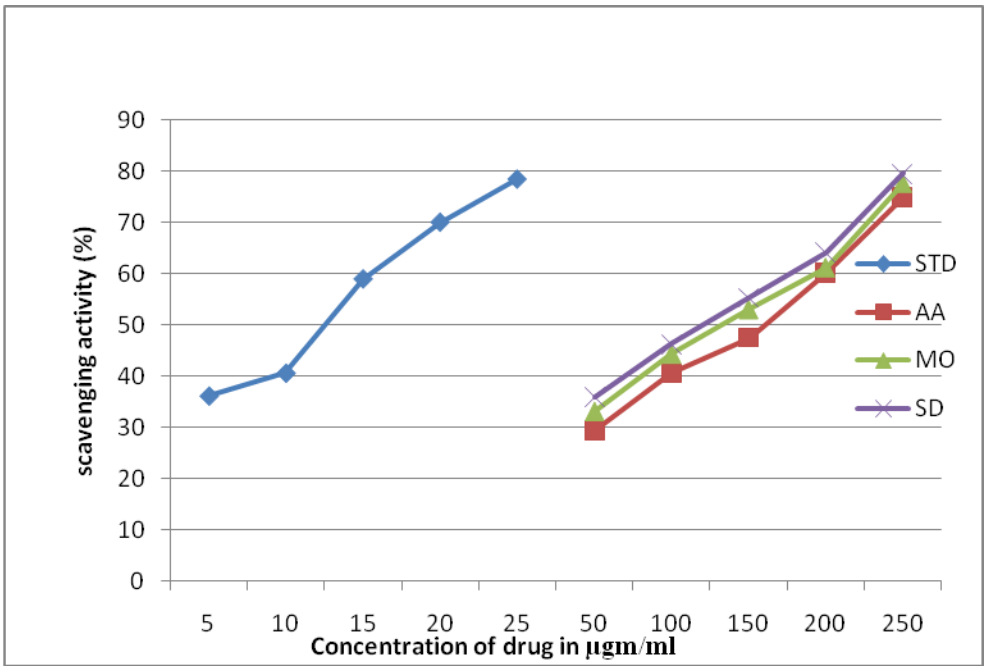

Fig 11. Scavenging of hydrogen peroxide

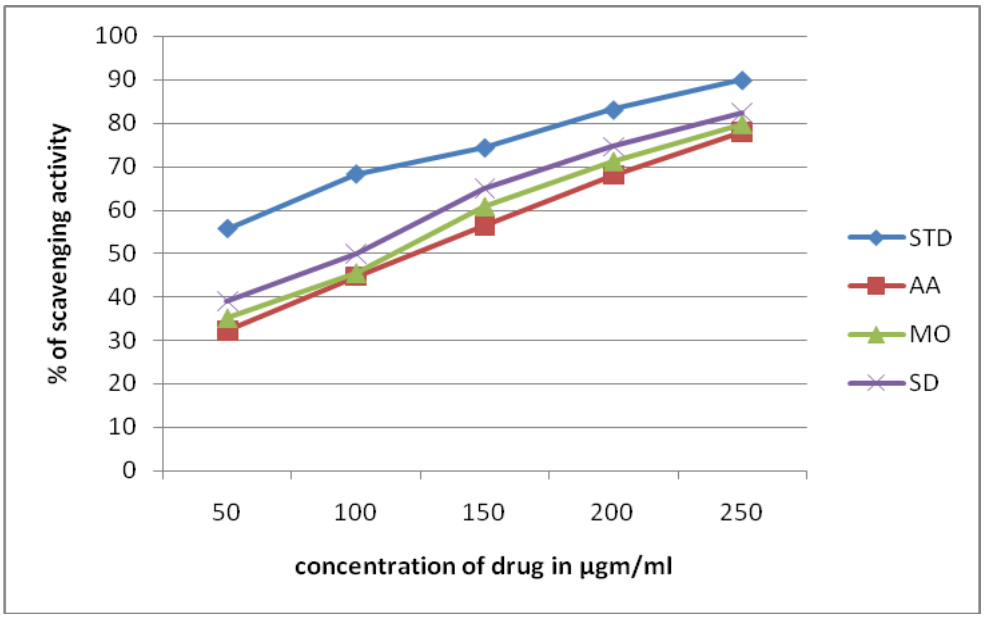

Fig 12. Reducing power determination

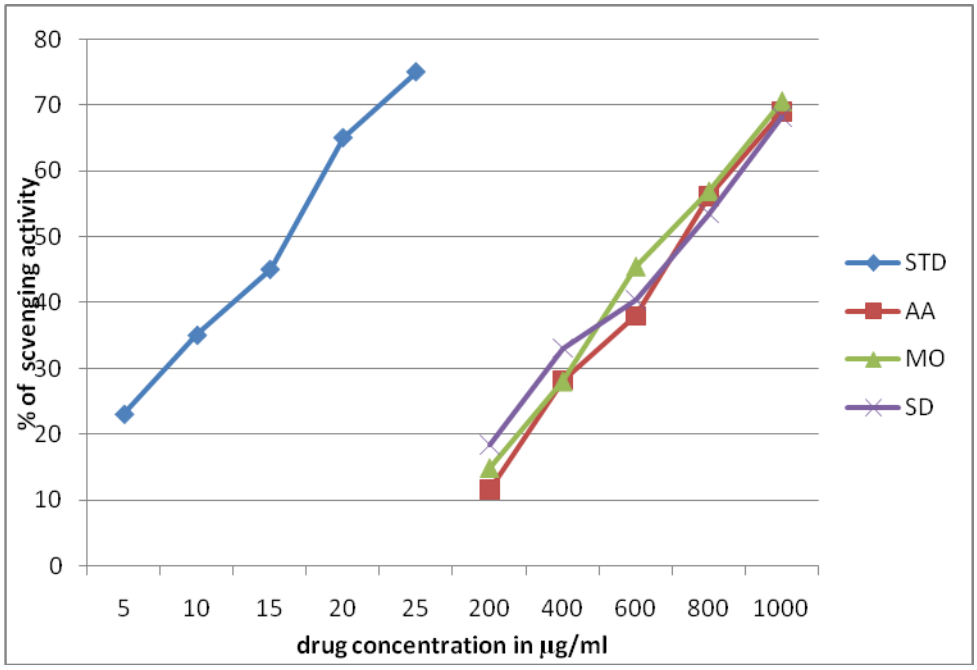

Fig 13. Nitric oxide scavenging activity

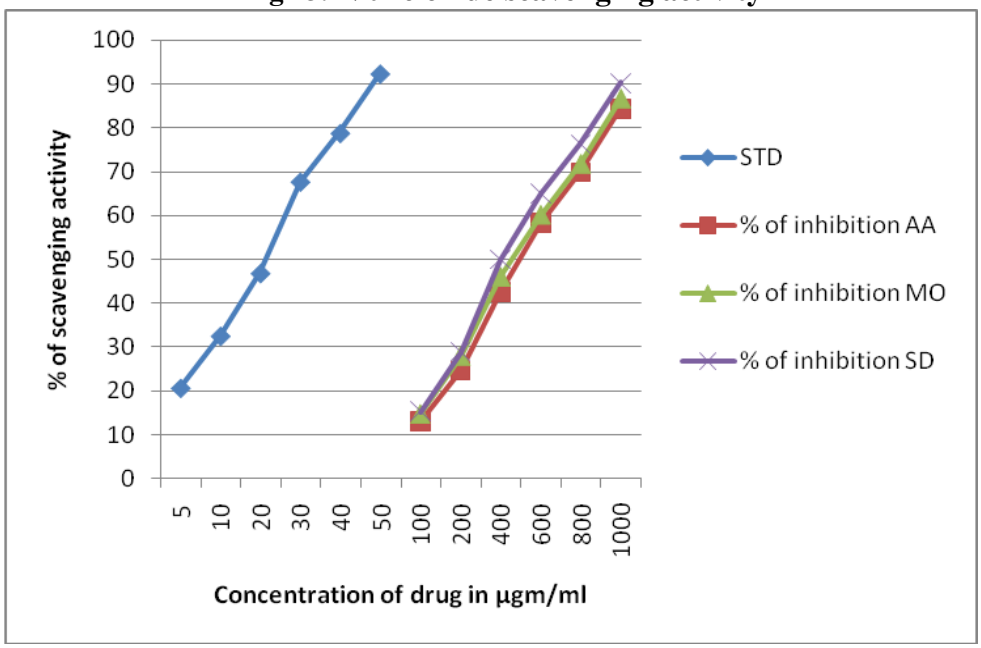

Fig 14. DPPH radical scavenging activity

Studies also revealed the augmented lipid peroxidation and lessened levels of antioxidant impending in kidneys of mice provided with ethylene glycol. The chief stone oxalate forming constituent has been perceived and documented to induce lipid peroxidation and cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes. Phenolic compounds present in *Glochidion velutinum* extracts may prevent the lipidperoxidation induced renal damage caused by calcium oxalate crystal setting up in the renal tubules. Consequently *Glochidion velutinum* extracts can prevent calciumoxalate crystal attachment as well as stone formation. Glochidion velutinum extracts treatment produced noteworthy decrease In MDA and increased GSH, SOD and CAT. These consequences indicate the protective effects of Glochidion velutinum extracts aligned with the oxidative modify hindered through ethylene glycol. These traits have been attributed to the triterpenes. Lupeol and polyphenolic compounds like quercetin may present in Glochidion velutinum extracts. Thus the consequences reveal that the Glochidion velutinum extracts hold a robust anti-oxidant and anti-urolilithiatic action similar to pomegranate juice.

In the present study, long term delivery of 1% (v/v) ethylene glycol solution to the Wistar rats consequenceed in hyperoxaluria. Urinal concentration of the an assortment of ions investigated varied drastically, following ethylene glycol treatment in the lithiatic groups., , Uric acid, Oxalate, Calcium, Creatinine and Phosphate excretion were notably increased on day 14^{th}& 28^{th} respectively for GROUP-II following ethylene glycol treatment. Treatment with Ethanolic and aqueous extract of Glochidion velutinum abridged the excretions notably on 14^{th} day of treatment and more abridged on 28^{th} day, like standard. In GP_1 normal rats the magnesium emission was assessed as 4.20±0.52 mg/dl/24hr, 4.42±0.58 mg/dl/24hr on 14^{th}& 28^{th} day. Contrary to this, in GROUP-II lithiatic control rats, the magnesium level in urine gradually lessened for ethylene glycol treatment on the 14^{th}& 28^{th} day.Subsequent delivering of the extract superior the magnesium emission notably on 14^{th} day & 28^{th} day.

In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased notably in GROUP-II (Lithiatic control) following ethylene glycol treatment, Treatment with Ethanolic and extract of *Glochidion velutinum* at a dose of 200mg/kg reduce the all above mentioned parameters notably. On the contrary the magnesium

levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol treatment**.** Subsequent to management with Ethanolic and extract of Glochidion velutinum at a dose of 200mg/kg the magnesium level was restored near to normal and standard levels.
In stone induced models, the subsequent modifications were observed, damaged epithelial cells at the inner layer of the tubules, Dilatation of the tubules and Presence of crystals. Score were agreed according to the severity of changes in the tubules. Sections of kidney from animals treated with ethylene glycol depicted large quantity of microcrystal deposition and stern relaxation of tubules and throng tubule interstitial inflammatory infiltration with lesion area > 40% (score3). On the other hand, kidney segment of animals subjected with extract depicts obvious dilation of many tubules and tubule interstitial provocative penetration with abrasion area < 40% (score 2)

As customary medicines are usually taken by the oral route, same route of administration was used for evaluation of antilithiatic effect of the Ethanolic and aqueous extract of three drugs. In the present study, male rats were chosen to persuade urolithiasis because the urinal system of male rats resembles that of humans and also earlier studies have depictn that the amount of stone deposition in female rats was notably less. Urinal super saturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that in response to 14 day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate The biochemical mechanisms for this process are related to an augment in the urinal concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate Similar consequences have been obtained when rats were treated with ethylene glycol and ammonium oxalate. Therefore, this model was used to evaluate the antilithiatic effect of Ethanolic and Aqueous extract of Glochidion velutinum at a dose of 200mg/kg aligned with urolithiasis.

In the present study oxalate and calcium excretion progressively increased in calculi-induced animals (GROUP-II), since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stones than hypercalciuria, and the changes in urinal oxalate levels are relatively much more important than those of calcium . Increased urinal calcium is a factor

favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth.However Ethanolic and Aqueous extract of Glochidion velutinum at a dose of 200mg/kg lowered the levels of oxalate as well as calcium excretion.

An increase in urinal phosphate is observed in calculi induced rats (GROUP-II). Increased urinal phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which is epitaxially, induces calcium oxalate deposition. Treatment with Ethanolic and aqueous extract of Glochidion velutinum at a dose of 200mg/kg restored phosphate level, thus reducing the risk of stone formation.The increases in urinal uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Treatment with Ethanolic and aqueous extract of Glochidion velutinum at a dose of 200mg/kg lowered the excretion of uric acid and reduces the risk of stone formation.

Super saturation, a step in the pathogenesis of nephrolithiasis, occurs when substances that make up the stone are found in the high concentration in urine, when urine volume decreases, and when urinal concentration of chemicals that inhibit stone formation decreases. Inhibitors of crystallization include citrate, magnesium, phosphate; nephrocalcin etc. Low urinal magnesium content is a common feature in stone formers. A similar condition was observed in the (GROUP-II) rats. Treatment with Ethanolic and Aqueous extract of Glochidion velutinum at a dose of 200mg/kg elevated the urinal magnesium level, and thus, abridged the propensity to crystallize, thereby creating an ambience unfavorable for precipitation.Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers .A high urinal colloidal concentration favours crystal growthSuch a condition was observed with ethylene glycol treated rats, in this study. Administration of the Ethanolic and Aqueous extract of Glochidion velutinum abridged the urinal protein excretion in the treated group rats, and hence minimizes the conditions favorable for crystal growth.

In urolithiasis, the Glomeruli Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinal system. Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated .Also increased lipid peroxidation and lessened levels of antioxidant potential have been perceived and documented in the kidneys of rats supplemented with a calculi- producing diet (CPD). Elevated oxalate has been perceived and documented to induce lipid peroxidation and to cause renal tissue damage by reacting with poly unsaturated fatty acids in the cell membrane. In calculi- induced rats (GROUP-II), marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid. However, the prophylactic treatment with Ethanolic and Aqueous extract causes diuresis and has tens the process of dissolving the preformed stones and prevention of new stone formation in the urinal system.Increase in calcium and oxalate levels in the renal tissue of EG-treated rats were observed. Prophylactic treatment with Ethanolic and Aqueous extract of *Glochidion velutinum* suppresses this increase in intracellular calcium. Several studies perceived and documented those Flavonoids, polyphones and triterpenes have anti-inflammatory and antioxidant effects. It can be expected that antilithiatic activity might be through an antioxidant activity and free radical scavenging principle.

Microscopic examination of kidney sections derived from ethylene glycol induced urolithiasis rats depicted polymorphic irregular crystal deposits inside the tubules which cause dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with Ethanolic and Aqueous extract of *Glochidion velutinum* lessened the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes.

This study also revealed the increased lipid per oxidation and lessened levels of antioxidant potential in kidneys of rats supplemented with ethylene glycol. Oxalate, the chief stone forming constituent, has been perceived and documented to induce lipid peroxidation and cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes. Phenolic compounds present in the extracts may prevent the lipid peroxidation induced renal damage caused by calcium oxalate crystal deposition in the kidney. Hence these extracts can prevent calcium oxalate crystal attachment as well as stone formation. The extracts treatment produced

noteworthy decrease in MDA and increased GSH, SOD and CAT these consequences indicate the protective effects of Glochidion velutinum extracts aligned with the oxidative changes induced by ethylene glycol. These properties have been attributed to the triterpenes. Lupeol and polyphenolic compounds like quercetin present in *Glochidion velutinum* extracts. Thus, the consequences reveal that the three extracts posses a potent antiurolilithiatic and antioxidant activity.In vivo antioxidant activity ethylene glycol treatment increased MDA ($P<0.01$) and lessened GSH ($p<0.01$) SOD ($p<0.01$) and CAT (0.01) levels in control animals.Aqueous and Ethanolic extracts of *Glochidion velutinum* produced noteworthy ($p<0.001$) reduction in MDA and increased GSH and antioxidant enzyme likes SOD and CAT compared to standard group cystone In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to e wistar rats consequenceed in hyperoxaluria. Urinal concentration of the an assortment of ions investigated varied drastically, following ethylene glycol treatment.

7.5 Effect of Glochidion Velutinum on Urinal Parameterson Day 14&28

The oxalate excretion was 15.80± 1.83 mg/dl/24hr & 16.30± 1.50mg/dl/24hr on day 14^{th}& 28^{th} respectively for GP_1. It increased notably to 32.6± 3.43mg/dl/24hr & 48.20± 4.95mg/dl/24hr ($P < 0.001$) on day 14^{th}& 28^{th} day in GROUP-II following ethylene glycol treatment. Treatment with Ethanolic and Aqueous extract of Glochidion velutinum at a dose of 200mg/kg abridged the oxalate excretion notably to; ($P<0.01$) on 14^{th} day treatment. Likewise on 28^{th} day, treatment with this extractsabridged the oxalate excretion notably.

The urinal calcium excretion was 5.63±0.54mg/dl/24hr& 6.15±0.70mg/dl/24hr on day 14^{th}& 28^{th} respectively for GP_1.It increased notably to 20.25± 1.98mg/dl/24hr &22.15± 1.60mg/dl/24hr ($P < 0.01$) on day 14^{th}& 28^{th} day in GROUP-IIfollowing ethylene glycol treatment. The calcium excretion was notably abridged totreatment with mthanolic and aqueous extract of Glochidion velutinum at a dose of 200mg/kg reduce the calcium excretion notably ($P < 0.01$) on 14^{th} day treatment likewise on 28^{th} day calcium excretion was notably abridged in rats nearer to that of the standard drug treated rats respectively.

Likewise phosphate 73.60±4.26mg/dl/24hr, 78.66±4.26mg/dl/24hr and creatinine 1.56± 0.14 mg/dl/24hr,1.86±0.24 mg/dl/24hr excretion values gradually increased in GROUP-II on the 14^{th}&

28th day. However in grouped treated animals these elevated values were brought down, regarding creatinine in these elevated values were brought down respectively.

Likewise urinal protein and uric acid concentration increased following ethylene glycol treatment in GROUP-II and it reached maximum excretion respectively on the 14^{th}& 28^{th} day. On treatment with Ethanolic and aqueous extract of Glochidion velutinum at a dose of 200mg/kg (GP_3 to GP_8) the protein and uric acid excretion was restored to near normal limits in (GP_3 to GP_8)for protein when comparing with the standard drug treated animals ($P<0.001$).

In GP_1 normal rats the magnesium excretion was estimated as 4.20±0.52 mg/dl/24hr, 4.42±0.58 mg/dl/24hr on 14^{th}& 28^{th} day. Contrary to this, in GROUP-II lithiatic control rats, the magnesium level in urine gradually lessened to 0.98±0.14 mg/dl/24hr 1.35± 0.11 mg/dl/24hr following ethylene glycol treatment on the 14^{th}& 28^{th} day .Subsequent administration of the extract enhanced the magnesium excretion notably comparing to the standard drug($P< 0.01$) respectively on 14^{th} day & 28^{th} day.

7.6 Effect of glochidion velutinum on serum parameters on day 28

In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased notably in GROUP-II (Lithiatic control) following ethylene glycol treatment, Treatment with Ethanolic and aqueous extract of Glochidion velutinum at a dose of 200mg/kg reduce the all above mentioned parameters notably. On the contrary the magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol treatment**.** After treatment with Ethanolic and Aqueous extract of Glochidion velutinum at a dose of 200mg/kg the magnesium level was restored near to normal levels.

7.7 Effect of *glochidion velutinum* on histopathological studies on day 28

In stone induced models, the following changes were noted

1. Damaged epithelial cells at the inner layer of the tubules.
2. Dilatation of the tubules
3. Presence of crystals in the tubules

Scores were given according to the severity of changes in the tubules. Sections of kidney from animals treated with ethylene glycol depicted large quantity of microcrystal deposition and severe dilation of most tubules and mass tubulointerstitial inflammatory infiltration with lesion area > 40% (score3). However, kidney sections of animals treated with Ethanolic and Aqueous extract of Glochidion velutinum at a dose of 200mg/kg depicts obvious dilation of many tubules and tubulointerstitial inflammatory infiltration with lesion area < 40% (score 2)

In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to Wistar rats consequenceed in hyperoxaluria. Urinal concentration of the an assortment of ions investigated varied drastically, following ethylene glycol treatment.

The oxalate excretion was increased on day 14^{th}& 28^{th} respectively for GP_1. It increased notably on day 14^{th}& 28^{th} day in GROUP-II following ethylene glycol treatment. Treatment with Ethanolic and aqueous extract of Glochidion velutinum at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg (GP_3 to GP_9) abridged the oxalate excretion notably on 14^{th} day treatment. Likewise on 28^{th} day, treatment.

The urinal calcium excretion was 5.63±0.54mg/dl/24hr& 6.15±0.70mg/dl/24hr on day 14^{th}& 28^{th} respectively for GP_1.It increased notably on day 14^{th}& 28^{th} day in GROUP-II following ethylene glycol treatment. The calcium excretion was notably abridged to treatment with Ethanolic and aqueous extract of *Glochidion velutinum* at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg reduce the calcium excretion notably in rats respectively. Likewise phosphate and creatinine excretion values gradually increased in GROUP-II on the 14^{th}& 28th day. However in grouped treated animals these elevated values were brought down on 14^{th} day and on 28^{th} day. However, regarding creatinine in these elevated values were brought down.

Likewise urinal protein and uric acid concentration increased following ethylene glycol treatment in GROUP-II and it reached high on the 14^{th}& 28^{th} day. On treatment with Ethanolic and aqueous extract of Glochidion velutinum at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg the protein and uric acid excretion was restored to near normal limits in on 28^{th} day.

In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased notably in GROUP-II (Lithiatic control) following ethylene glycol treatment, Treatment with Ethanolic and aqueous extract of Glochidion velutinum reduce the all above mentioned parameters notably. On the contrary the magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol treatment**.** After treatment with Ethanolic and aqueous extract of Glochidion velutinum at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg the magnesium level was restored near to normal levels.

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Scores were given according to the severity of changes in the tubules. Sections of kidney from animals treated with ethylene glycol GROUP-II depicted large quantity of microcrystal deposition and severe dilation of most tubules and mass tubulointerstitial inflammatory infiltration with lesion area > 40% (score3). However, kidney sections of animals treated with Ethanolic and Aqueous extract of Glochidion velutinum at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg depicts obvious dilation of many tubules and tubulointerstitial inflammatory infiltration with lesion area < 40% (score 2) in GP:71to77.

In the present study, male rats were selected to induce urolithiasis because the urinal system of male rats resembles that of humans and also the amount of stone deposition in female rats was notably less. Evidence in previous studies indicated that in response to 14 day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate. The biochemical mechanisms for this process are related to an increase in the urinal concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate In the present study oxalate and calcium excretion progressively increased in calculi- induced animals (GROUP-II), since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis

of renal stones than hypercalciuria, and the changes in urinal oxalate levels are relatively much more important than those of calcium. Increased urinal calcium is a factor favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth. However extracts of Glochidion velutinum at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg lowered the levels of oxalate as well as calcium excretion.

An increase in urinal phosphate is observed in calculi induced rats (GROUP-II). Increased urinal phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which is epitaxially induces calcium oxalate deposition. Treatment with the extracts of Glochidion velutinum at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg restored phosphate level, thus reducing the risk of stone formation.

The increases in urinal uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Treatment with extracts of Glochidion velutinum at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg lowered the excretion of uric acid and reduces the risk of stone formation.Low urinal magnesium content is a common feature in stone formers. A similar condition was observed in the (GROUP-II) rats. Treatment with extracts of Glochidion velutinum at a dose of 200mg/kg and Cystone herbal tablet at a dose of 100mg/kg elevated the urinal magnesium level, and thus, abridged the propensity to crystallize, thereby creating an ambience unfavourable for precipitation.

Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers .A high urinal colloidal concentration favours crystal growth. Such a condition was observed with ethylene glycol treated rats, in this study. Administration of the extracts of Glochidion velutinum at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg abridged the

urinal protein excretion in the treated group rats, and hence minimizes the conditions favourable for crystal growth.

In urolithiasis, the Glomeruli Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinal system. Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated. Also increased lipid per oxidation and lessened levels of antioxidant potential have been perceived and documented in the kidneys of rats supplemented with a calculi- producing diet (CPD). Elevated oxalate has been perceived and documented to induce lipid per oxidation and to cause renal tissue damage by reacting with poly unsaturated fatty acids in the cell membrane.

Microscopic examination of kidney sections derived from ethylene glycol induced urolithiasis rats depicted polymorphic irregular crystal deposits inside the tubules which cause dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with extracts of Glochidion velutinum and cystone herbal tablet lessened the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes. In stone induced models, the following changes were noted, damaged epithelial cells at the inner layer of the tubules, dilatation of the tubules and presence of crystals in the tubules. Scores were given according to the severity of changes in the tubules.

In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to the Wistar rats consequenceed in hyperoxaluria. Urinal concentration of the an assortment of ions investigated varied drastically, following ethylene glycol treatment in the lithiatic control. The oxalate, Calcium, Uric acid, Creatinine and Phosphate excretion were notably increased on day 14^{th}& 28^{th} respectively for GROUP-II following ethylene glycol treatment. Treatment with Ethanolic and Aqueous extracts of Glochidion velutinum abridged the excretions notably on 14^{th} day of treatment and more abridged on 28^{th} day, like standar. In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased notably in GROUP-II (Lithiatic control) following ethylene glycol treatment. Treatments with Ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200mg/kg reduce the all above mentioned parameters notably. On the contrary, the

magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol treatment**.** After treatment with Ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200mg/kg the magnesium level was restored near to normal and standard levels.

In the present study, oxalate and calcium excretion progressively increased in calculi-induced animals (GROUP-II), since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stones than hypercalciuria, and the changes in urinal oxalate levels are relatively much more important than those of calcium. Increased urinal calcium is a factor favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth. However Ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200mg/kg lowered the levels of oxalate as well as calcium excretion.

An increase in urinal phosphate is observed in calculi induced rats (GROUP-II). Increased urinal phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially, induces calcium oxalate deposition. Treatment with Ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200mg/kg restored phosphate level, thus reducing the risk of stone formation. The increases in urinal uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Treatment with Ethanolic and aqueous extract of Glochidion velutinum lowered the excretion of uric acid and has abridged the risk of stone formation.

Super saturation, a step in the pathogenesis of nephrolithiasis, occurs when substances that make up the stone are found in the high concentration in urine, when urine volume decreases, and when urinal concentration of chemicals that inhibit stone formation decreases. Inhibitors of crystallization include citrate, magnesium, phosphate, nephrocalcium etc.Low urinal magnesium content is a common feature in stone formers, and similar condition was observed in the (GROUP-II) rats. Treatment with Ethanolic and aqueous extracts of *Glochidion*

velutinum at a dose of 200mg/kg elevated the urinal magnesium level, and thus, abridged the propensity to crystallize, thereby creating an ambience unfavorable for precipitation. Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers. A high urinal colloidal concentration favours crystal growth. Such a condition was observed with ethylene glycol treated rats, in this study. Administration of the Ethanolic and Aqueous extracts of *Glochidion velutinum* abridged the urinal protein excretion in the treated group rats, and hence it has minimized the conditions favourable for crystal growth

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In urolithiasis, the Glomeruli Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinal system (Table no. 11). Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated. Also increased lipid per oxidation and lessened levels of antioxidant potential has been perceived and documented in the kidneys of rats supplemented with a calculus- producing diet (CPD). Elevated oxalate has been perceived and documented to induce lipid peroxidation and to cause renal tissue damage by reacting with poly unsaturated fatty acids in the cell membrane. In calculi- induced rats (GROUP-II), marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid. However, the prophylactic treatment with Ethanolic and Aqueous extracts caused diuresis and has tensed the process of dissolving the preformed stones and prevention of new stone formation in the urinal system. Increase in calcium and oxalate levels in the renal tissue of EG-treated rats were observed. Prophylactic treatment with Ethanolic and Aqueous extracts of *Glochidion velutinum* suppressed this increase in intracellular calcium.

Microscopic examination of kidney sections derived from ethylene glycol induced urolithiasis rats depicted polymorphic irregular crystal deposits inside the tubules which cause dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with Ethanolic and Aqueous extracts of Glochidion velutinum lessened the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes.

This study also revealed the increased lipid per oxidation and lessened levels of antioxidant potential in kidneys of rats supplemented with ethylene glycol. Oxalate, the chief stone forming constituent, has been perceived and documented to induce lipid peroxidation and

cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes. Phenolic compounds present in the extracts may prevent the lipidperoxidation induced renal damage caused by calcium oxalate crystal deposition in the kidney. Hence these extracts can prevent calcium oxalate crystal attachment as well as stone formation. The extracts treatment produced noteworthy decrease in MDA and increased GSH, SOD, and CAT these consequences indicate the protective effects of Glochidion velutinum extracts aligned with the oxidative changes induced by ethylene glycol. Thus, the consequences revealed that the three extracts posses a potent antiurolilithiatic and antioxidant activity. In vivo antioxidant activity ethylene glycol treatment increased MDA ($P<0.01$) and lessened GSH ($P<0.01$) SOD ($P<0.01$) and CAT (0.01) levels in control animals. Aqueous and Ethanolic extracts of Glochidion velutinum produced noteworthy reduction ($P<0.001$) in MDA and increased GSH and antioxidant enzyme likes SOD and CAT compared to standard group Cystone.

7.8 Invitro antioxidant activity

The antioxidant activity of the Ethanolic extract was estimated by five methods. DPPH radical scavenging activity, Reducing power determination, Scavenging of hydrogen peroxide, Assay of nitric oxide scavenging activity and compared with different standards like Ascorbic acid, Quercetine and Epicatechine. Three plants extracts depicted good antioxidant activity. The IC50 values for all methods were calculated and documented.

The flavonoids are a heterogeneous group of phenol compound present in the plant world.The functions of flavonoids in plants include pigmentation, protection aligned with UV light and microorganisms, defense aligned with grazing animals or a regulatory function for enzymes and signal substances for nitrogen fixing bacteria. The flavonoids are abundant in the human diet. They are principally found in fruits, vegetables and popular drinks, such as red wine, tea, coffee and beer. Many flavonoids, purified from medicinal plants are herbs used in the practice customary medicine, are endowed with biological effects. Flavonoids may directly scavenge some radical species by acting as chain breaking antioxidants or they may cycle other chain-breaking antioxidants such as tocopherols by donating a hydrogen atom to tocopherol radical. Transition mineral such as ferric and copper are important pro oxidants and some flavonoids can chelate divalent metal ions, hence preventing free radical formation.

Glochidion velutinum are having antioxidant activity. It was evaluated by five methods. In the Hydrogen peroxide scavenging activity method the IC50 values are calculated and compared with the standard Ascorbic acid , it depicts 140 µg of *Glochidion velutinum* of is equivalent to that of 12.5 µg of ascorbic acid. Flavonoids are phenolic compounds, present in several plants, which inhibit lipid per oxidation and lipoxygenases in vitro and in presence of free metal ion (Fe^{3+}).By the reducing power activity method the IC50 values are calculated and compared with the standard Epicatechine. The consequence depicts 114 µg *Glochidion velutinum* is equivalent to 12.5 µg of Epicatechine activity .

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of an assortment of physiological processes. Excess concentration of nitric oxide is implicated in the cytotoxic effects observed in an assortment of disorders like AIDS, cancer, Alzheimer's and arthritis. Oxygen reacts with the excess NO to generate nitrite and peroxy nitrite anions, which act as free radicals. From consequences of Nitric oxide method, it proved that the aqueous leaf extract of *Glochidion velutinum* has effective anti oxidant activity. These extract compete with oxygen to react with NO and thus inhibit the generation of the nitrite and peroxy nitrite anions. In the Nitric oxide scavenging activity method the IC50 values are calculated and compared with the standard Quercetine IC50 value. It depicts 294.16 µg of *Glochidion velutinum* of SD is equivalent to 25 µg of Quercetine.

DPPH is relatively stable nitrogen centred free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitablereducing agents as a consequence of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending onthe number of electrons taken up. Substances capable of donating electrons/hydrogen atomsare able to convert DPPH (Purple) into their no radical form 1, 1-diphenyl-2- picrylhydrazine (Yellow), a reaction which can be followed spectrophotometrically. Free radicalscavenging activity of the methanol leaf extract of *Glochidion velutinum* is concentration dependent, as theconcentration of the test compounds increases, the radical scavenging activity increases. In the DPPH scavenging activity method the IC50 values are calculated and it gives a good consequence of 384µg of *Glochidion velutinum* is equivalent to 24µgof Ascorbic acid.

8. SUMMARY & CONCLUSION

This present study evaluated *Glochidion velutinum* for urolithiasis and related problems in human. As conventional medicines are typically taken by the oral route, same course of administration was used for assessment of antilithiatic consequence of the extracts of *Glochidion velutinum* at a dose of 200mg/kg and Cystone herbal tablet at a dose of 100mg/kg aligned with ethylene glycol induced urolithiasis in rats. In the present study, male rats were selected to persuade urolithiasis because the urinal system of male rats resembles that of humans and also the quantity of stone deposition in female rats was drastically fewer. Substantiation in preceding studies indicated that in response to 14 day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi encompassed mainly of calcium oxalate. The biochemical mechanisms for this progression are related to a raised in the urinal concentration of oxalate. Stone development in ethylene glycol fed animals is caused by hyperoxaluria, which causes augmented renal retention and emission of oxalate. In the present study oxalate and calcium excretion progressively augmented in calculi- induced animals, since it is customary that hyperoxaluria, is a far more risk reason in the pathogenesis of renal stones than hypercalciuria, and the changes in urinal oxalate levels are comparatively much more important than those of calcium. Augmented urinal calcium is a feature favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and consequent crystal growth. Conversely, extracts of *Glochidion velutinum* treated animals lowered the levels of oxalate as well as calcium excretion.

In the present study, *Glochidion velutinum* are having good antioxidant and antiurolithiatic activity, it was proved obviously in this juncture. Meticulous percevings of the patho-physiology of illness and method of action of these herbal medicines have great importance in improvement of effective and safe antiurolithiatic agent. The antioxidant action was calculated as free radical scavenging activity technique, Nitric oxide scavenging, DPPH method, Reducing control determination technique, Hydrogen peroxide method. All the methods depict good response due to thepresence of phenolic compounds and flavonoids in three species. The herbal drugs exert their urolithiatic consequence by varying the ionic content of urine lessening the ca+ and oxalate ion strength or escalating magnesium and citrate excretion and also with diuretic activity. In this respect this information provides a fruitful area

for scientific research by willing investigators.An attempt may be made to develop new herbal formulation to treat Kidney stone by *Glochidion velutinum* plants. From this present study we can conclude by using this *Glochidion velutinum,* we can go for herbal formulation development to treat Kidney stone.

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